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The Observation of Arbuscular Mycorrhizal Roots in Horticultural Plants

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To determine the degree of variability among the host plant species in their abilities to become colonized by arbuscular mycorrhizal fungi (AMF), the inoculum for AMF was collected from the various sites in Korea and was inoculated to the three horticultural plants; *Tagetes patula*, *Torenia fournieri*, and *Salvia splendens*. After 4-month growth under greenhouse, mycorrhizal root colonization rates and spore density were measured. The roots of *T. patula* showed higher colonization rate than both plants of *T. fournieri* and *Salvia splendens*. The mycorrhizal root colonization was influenced by both of the AM fungal inoculum and the host species or their interactions. The combination of the host and fungal species was suggested to be important for the application of AMF to horticultural crops.

KEYWORDS: **Arbuscular mycorrhiza, Horticulture, *Savia splendes*, *Tagetes patula*, *Torenia fournieri***

Arbuscular mycorrhizal (AM) fungi have an obligate mutualistic association with the most terrestrial plant species (Trappe, 1987). Mycorrhizal plants have higher uptake of water and some inorganic nutrients, in particular phosphorus, improve the yield and are more tolerant of many kinds of environmental stresses and pathogens than are nonmycorrhizal plants. Potential use of mycorrhizal fungi in agriculture has received much attention to improve yields and reduce use of chemical fertilizers in the past decades. However, previous studies have shown difficulties to colonize mycorrhizal fungi in horticultural plant roots growing in soilless media (Nemec, 1987; Pedersen *et al.*, 1991). Only a few study has shown the successful use of AM fungi in horticultural productions (Miller *et al.*, 1986; Koid *et al.*, 1999). Ka *et al.* (1991) examined 36 horticultural plant species from a horticultural shop in Korea and they found that the 17 species were colonized by the arbuscular mycorrhizal fungi. However, There is only a few studies on mycorrhizal of horticultural plants in Korea.

Mycorrhizal propagules include hyphae, spores and colonized roots, and selection of AM inoculant depends on the growth condition of the plants. Soil inoculum has been commonly used because it contains all the components of mycorrhizal propagules. The ability of AM fungi to colonize the host plant roots may vary with morphological and physiological characteristics and ecological condition of their host plants. Also, the plants have varying degree of dependence on mycorrhizal association from the non-mycorrhizal plants to obligatory (Janos, 1980). Multiple AM fungal species can be colonized in a single root and mycorrhizal interaction has been thought to have little host specificity (Harley and Smith, 1983). However, several recent studies have shown that AM fungal species have different effect on host plants.

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The aim of the present study was to determine the degree of variability among the annual horticultural plant species in their ability to become colonized by mycorrhizal fungi when growing in a soilless potting medium with various AM inoculums.

Materials and Methods

Soils were collected from eight different sites where were growing four different host plants in Korea and were used as AM fungal inoculum (Table 1). A 100 m*l* of AM inoculum was mixed with the soilless potting medium, which were mixed with Perlite and Peatmoss (4 : 1, v/v). Three annual horticultural plants, *Tagetes patula*, *Torenia fournieri*, and *Salvia splendens*, were transplanted to the pots (30×10× 10 cm) and each treatment had the 4 replicate pots. The plants were maintained in a greenhouse and watered as needed. They were fertilized every 10 days from 8 weeks when they were started flowering with 500 m*l* of 1/4 strength of Hoaglands solution (Hoagland and Arnon, 1950) per pot.

Roots were collected after 4 months and stained by Trypan Blue (Koske and Gemma, 1989) and observed under

Table 1. Inoculum sources used for this study

Inoculum	Sites collected	Host plant species in the fields
A	Mt. Minjuji, Chungbuk	*Allium monanthum* Max
B	Trap cultured soil No. 4	*Capsium annuum* L.
C	Trap cultured soil No. 5	*Cassia mimosoides* var. *nomave Makino*
D	Cheongwon, Chungbuk	*Persicaria thunbergii* H. Gross
E	Mt. Minjuji, Chungbuk	*Allium monanthum* Max
F	Mt. Minjuji, Chungbuk	*Allium monanthum* Max
G	Mt. Minjuji, Chungbuk	*Allium monanthum* Max
H	Mt. Minjuji, Chungbuk	*Allium monanthum* Max

dissect and light microscopes. The percent mycorrhizal colonization rate within roots was measured by the Gridline intersection method (Giovannetti and Mosse, 1980). Soils were collected and homogenized manually. AM fungal spores were extracted from 10 g dry weight soil using wet-sieving or sucrose density gradient centrifugation (Daniels and Skipper, 1982). The extracted spores were observed and counted under a light microscope (40X). Only spores which appeared to be fresh (based on color, shape, surface conditions, and examination of spore contents) were counted. Data were analyzed by statistic variance using SPSS-WIN.

Results and Discussion

Inter- and intra-cellular hyphae, hyphal coils, arbuscules and vesicles were observed from all plant roots except the control nonmycorrhizal plants (Fig. 1). The roots of *T. patula* were heavily colonized by AM fungi, indicating staining intensity and the roots of *T. fournieri*, *S. splendens* were stained weakly. The staining intensity varies on the host species and the fungal species and mycorrhizal morphology is also influenced by the host root structure (Abbott, 1982; Brundrett and Kendrick 1990). In this study, the intensity of

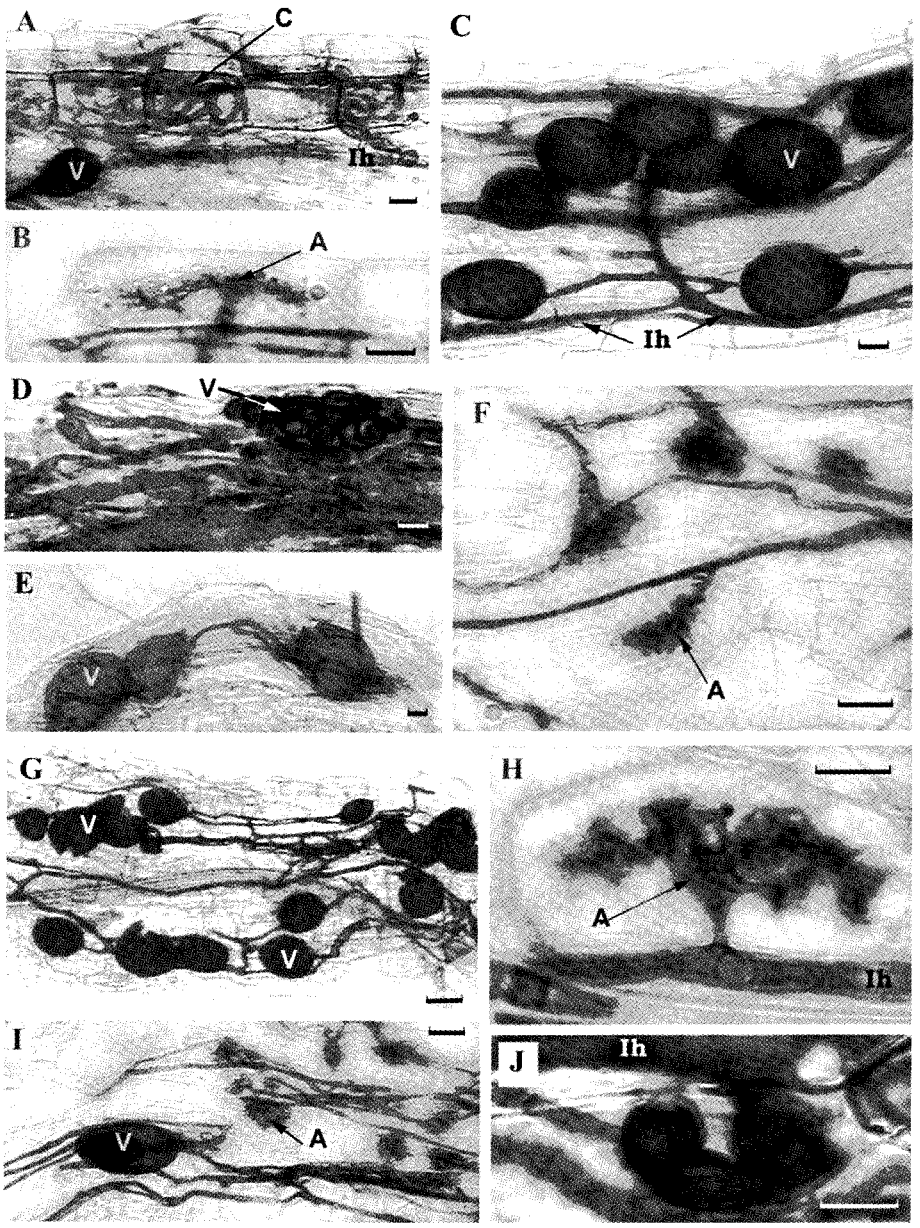

Fig. 1. The morphological characteristics of arbuscular mycorrhizae colonized in roots of three plant species. All samples were cleared and stained with Trypan Blue after 4 months growth in a greenhouse. (A-C) *Tagetes patula*, (D-F) *Torenia fournieri*, (G-I) *Savia splendes*. Abbreviations: A, arbuscules; V, vesicles; C, coiled hyphae; H, inter- or intra cellular hyphae. Scale bars represent 10 μm.

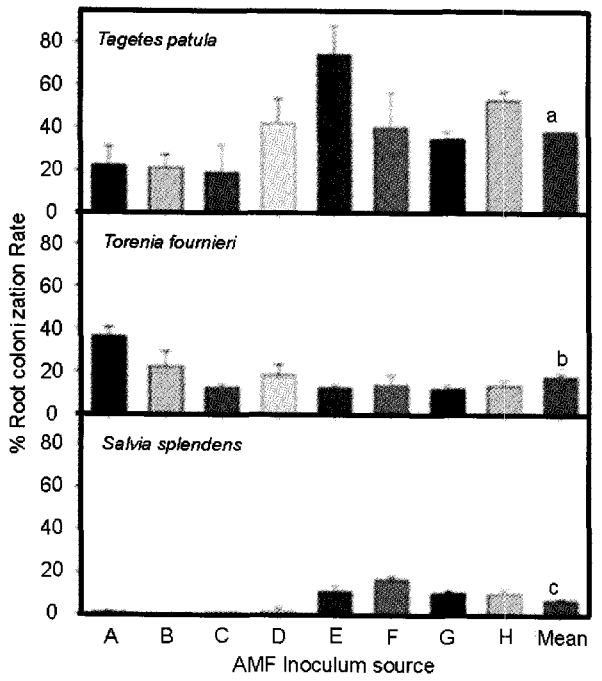

Fig. 2. Means (±1 SE, N = 4) of the total spore production per 10 g soil and percent root mycorrhizal colonization that is formed by the eight arbuscular mycorrhizal fungal inocula for three horticultural plant species after 4 month-growth under a greenhouse. Different letters on the graph represent differences among mean colonization rates of 3 plant species at the significance level, $P < 0.05$.

root colonization was but not likely to be related to the inoculum sources (Fig. 2) and could be due to the plant species, but not the fungal species colonized.

Mycorrhizal colonization of *T. patula*, *T. fournieri* and *S. splendens* ranged at the 0~92%, 0~54% and 0~19%, respectively, and showed significant difference among host plant species (Table 2). *T. patula* exhibited high level of root colonization while *S. splendens* showed low value and *T. fournieri* had intermediate level of colonization (Fig. 2). The result of two-way ANOVA for mycorrhizal root colonization showed the interactions between the host plant species and the inoculum sources (Table 2). Mycorrhizal effect on plant growth as well as root colonization depends on morphology, structure and their physiology of host plant species (Fitter 1987; Hetrick *et al.*, 1983).

Host species responded differentially to different AM fungal inocula (Fig. 2). The colonization rate of *T. patula* was significantly high in inoculum E and low in C while in *T. fournieri*, high in inoculum A, low in inoculum E. *S. splendens* was the highest colonization in inoculum E and no colonization in inoculum B. However, colonization rate in *S. splendens* showed similar trends with *T. patula*.

Table 2. Analysis of variance for the effects of host species and inoculum sources on mycorrhizal root colonization rate

Source of variation	df	F	P
Host	2	40.406	0.0001
Inoculum	7	3.119	0.0060
Host x Inoculum	14	3.898	0.0001

Total spores production varied from 0~191 spores per 10 g of dry soil (average 51 spores) and were significantly influenced by inoculum ($df = 7$, $F = 3.24$, $p = 0.015$). It was showed high sporulation in soil H and E and few spores in D (Fig. 2). Mean spore production was positively related to root colonization, but not significantly (Pearson correlation coefficient = 0.455, $P = 0.129$). There was no significant correlation between spore production and the root colonization of each host plant species. However, root colonization of *T. patula* among host plant species showed higher positive correlation coefficient value (0.489) with spore production than other host plant species in this study (*T. patula* = -0.212, *S. splendens* = 0.266). The high root colonization of *T. patula* (average 38.4%) might be related the spore production of AM fungi. However, these two parameters are not necessarily related because many other factors affect spore production (Allen and Allen, 1980, Giovannetti and Nicolson, 1983).

These results indicate that a plant species responded differentially to different fungal communities and fungal species influenced differentially on different host plant species. It suggests that it is important to select appropriate fungi when applying mycorrhizae to species horticultural plants. However, it is not possible to distinguish hyphae in the roots and soil morphologically at species level so far. Recently, several efforts has been made to identify these fungi using molecular techniques (Redecker *et al.*, 1997; Helgason *et al.*, 1998; van Tuinen *et al.*, 1998) and this will provide the most effective application of AM to agricultural plants and promise to reveal much about the biology of these symbiosis.

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